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NEPHROLOGY FORUM

Familial Mediterranean fever and amyloidosis

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Case presentation

A 28-year-old man was admitted to the Second Propedeutic Department of Medicine at the Aristotelian University of Thessaloniki for evaluation of the nephrotic syndrome. The patient was in excellent health until 2 years before admission, when he began to experience episodes of pleuritic chest pain that spread to the abdominal wall and to the back. The pain lasted 2 to 3 days with intervals of 10 to 30 days between attacks; it was intense and necessitated analgesics, usually aspirin, for relief. Low-grade fever had been documented during these episodes. Five months prior to admission, the patient had had a laparotomy at another hospital for suspected acute appendicitis but appendicitis was not found. Shortly after the operation, the patient developed edema, and proteinuria, up to 6 g/day, was discovered; renal function was normal. Prednisone, administered in a dose of 50 to 100 mg on alternate days, produced no improvement. One month prior to admission, a renal biopsy revealed amyloidosis (Figs. 1 and 2). Diuretics and a low-salt, high-protein diet were prescribed, but the edema did not improve.

The patient's family originated in Asia Minor and his sister already had been diagnosed in this hospital as having familial Mediterranean fever. She had no clinical evidence of nephropathy; her painful attacks had responded satisfactorily to colchicine.

On admission the patient complained of anorexia and a weight loss of 10 kg. Physical examination revealed the following: blood pressure, 120/80 mm Hg; pulse, 80; respirations, 16; and temperature 36.8° C. The head, eyes, ears, nose, and throat were unremarkable. The chest was clear to auscultation and percussion. The cardiac examination and peripheral pulses were normal. The abdominal and rectal examinations were unremarkable. Edema was present in the presacral area and below the knees. The neurologic examination was within normal limits. No skin rashes or other lesions were present.

Laboratory findings disclosed the following: hemoglobin, 14 g/dl; white blood cell count, 9400/mm^3 with a normal differential; platelets, 200,000/mm^3; serum creatinine, 1 mg/dl; BUN, 15 mg/dl; serum sodium, 138 mEq/liter; serum potassium, 3.8 mEq/liter; total carbon dioxide content, 26 mmol/liter; serum protein, 5.1 g/dl; serum albumin, 2.8 g/dl; serum cholesterol, 400 mg/dl; serum calcium, 9.5 mg/dl; serum phosphorus, 3.8 mg/dl; serum uric acid, 5.1 mg/dl. Serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) were normal. Fasting and 2-hour postprandial blood sugar concentrations were normal. An antinuclear antibody test and rheumatoid factor were negative. The 24-hour urinary protein excretion was 6.5 and 7.2 g in two consecutive collections. An intravenous urogram revealed kidneys of normal size with prompt bilateral function and no evidence of obstruction.

Renal amyloidosis secondary to familial Mediterranean fever was diagnosed, and the patient was treated with a low-salt, high-protein diet, 2 mg of colchicine, 200 mg of spironolactone, and 80 mg of furosemide or 100 mg of ethacrynic acid daily.

Two months later the patient had ankle edema but no further attacks of pain. The dose of colchicine was increased to 3 mg daily; however, because the patient began to have 3 or 4 bowel movements per day, the dose was subsequently reduced to 1.5 mg daily. By the eighth month of treatment, proteinuria had declined to less than 2 g/day. Diuretic treatment was discontinued, and a normal diet was prescribed. Over the subsequent 3 years, protein excretion ranged from 0.5 to 1.0 g/day. The patient's wife delivered a normal child during this interval. Four years after diagnosis, the dose of colchicine was reduced to 1 mg daily; soon thereafter, it was decreased to 0.5 mg daily because the patient was planning to father a second child.

Approximately 4.5 years after diagnosis, the physical examination was normal. Urinalysis revealed: specific gravity, 1.022; pH, 6; protein, 2+; no glucose; 5 to 8 white blood cells per high-power field; no red cells or casts were present. Serum protein electrophoresis showed the following: albumin, 61%; globulins, α_1, 2%; α_2, 7%; β, 17%; γ, 13%. Serum concentrations of immunoglobulins were: IgG, 680 mg/dl (normal, 800 to 2000 mg/dl); IgA, 157 mg/dl (normal, 150 to 450 mg/dl); and IgM, 194 mg/dl (normal, 50 to 200 mg/dl). Serum immune complexes were 0.061% O.D. (optical density) (normal, 0.075 ± 0.06% O.D.). Serum C3 concentration was 98 mg/dl (normal, 60 to 120 mg/dl); the C4 was 894 mg/dl (normal, 200 to 800 mg/dl). Assays for antinuclear, anti-DNA, and antimitochondrial antibodies all were negative.

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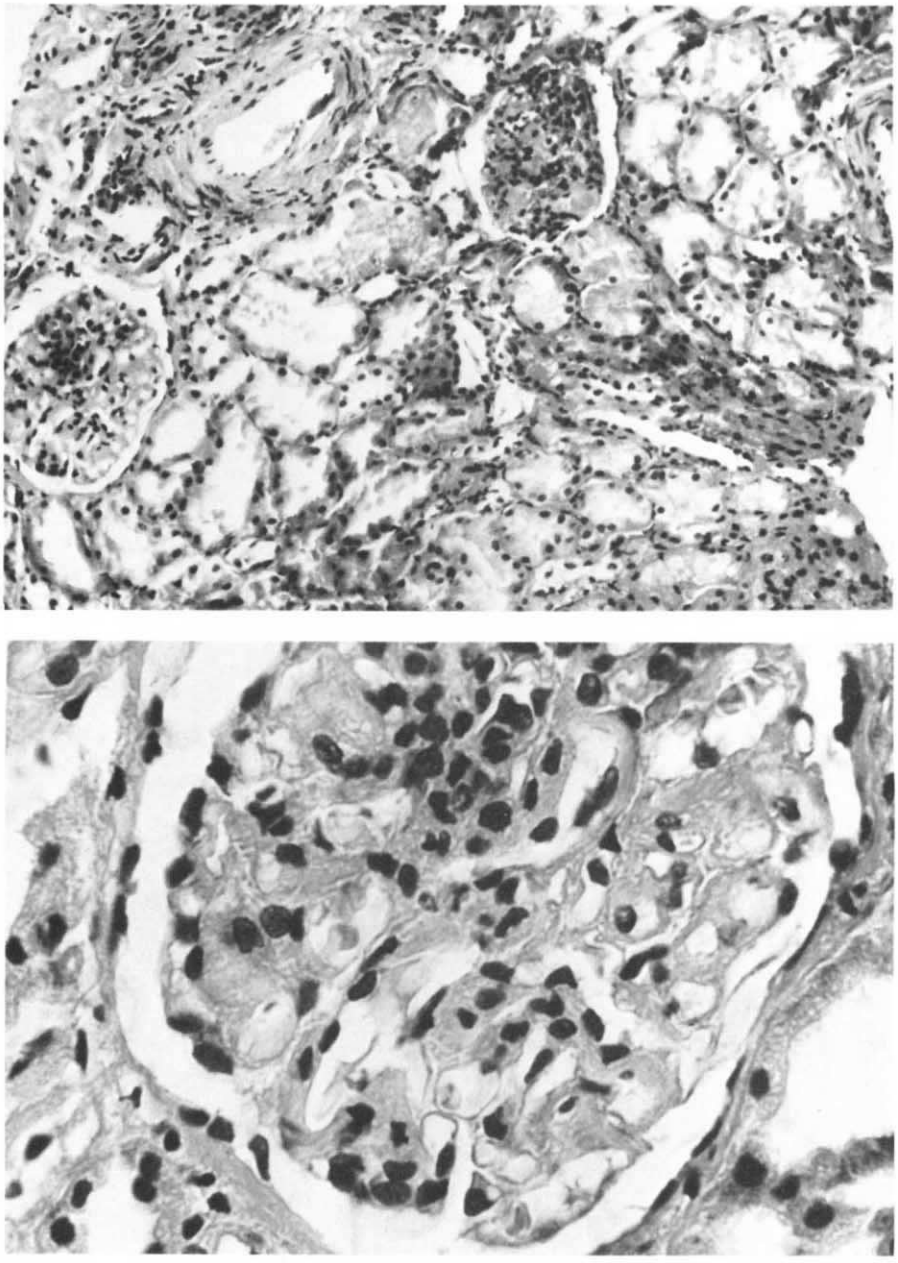
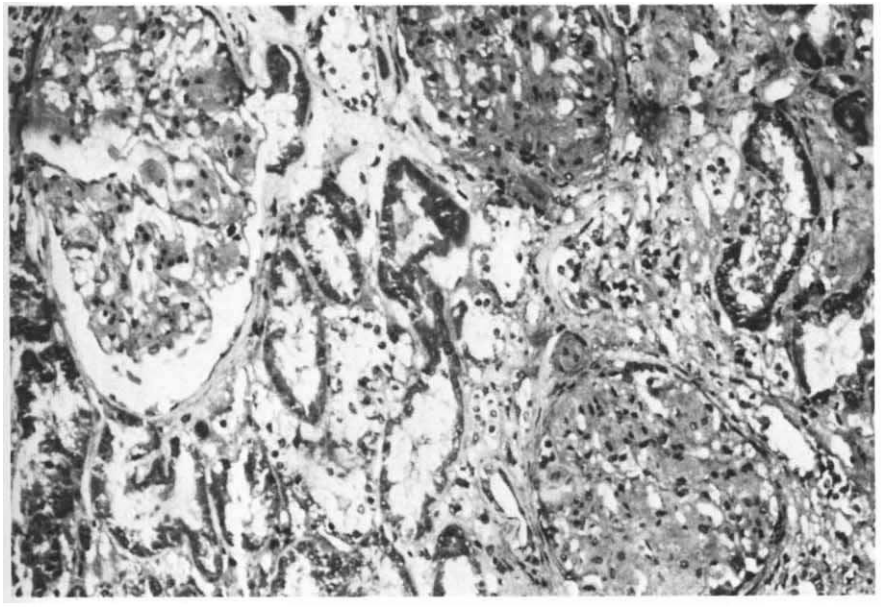


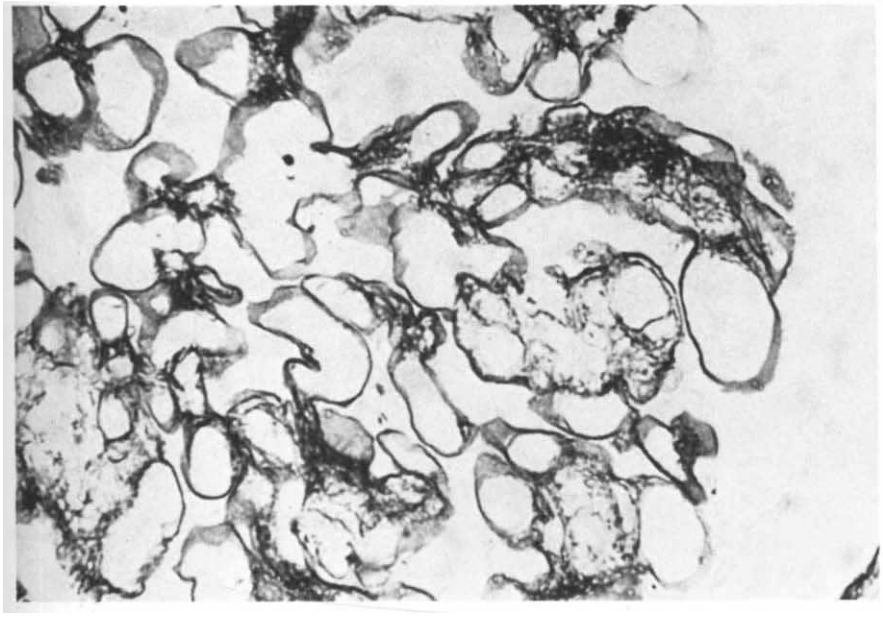
Figs. 1 and 2. *First biopsy (1974) showing fairly well preserved renal parenchyma with moderate amyloid deposits in the glomeruli and the arterial wall.*

Approximately 5 years after initiation of treatment, the patient was hospitalized for reevaluation. His general condition was excellent; blood pressure was normal and there was no peripheral edema. He continued to take only 0.5 mg of colchicine daily; he had been free of painful attacks. The laboratory findings revealed: hematocrit, 50%; white blood cell count, 8900/mm^3 with a normal differential; platelets, 200,000/mm^3; erythrocyte sedimentation rate, 30 mm/hr; serum creatinine, 1.3 mg/dl; BUN, 37 mg/dl; serum sodium, 142 mEq/liter; serum potassium, 4.8 mEq/liter; blood sugar, 68 mg/dl; SGOT and SGPT, normal; serum cholesterol, 197 mg/dl; serum protein, 6.2 g/dl; serum albumin, 3.7 g/dl. The 24-hour urinary protein excretion was 1.8 and 1.0 g in two consecutive collections. A second percutaneous renal biopsy was performed (Figs. 3 and 4), which will be discussed later.

Discussion

DR. PANAGIOTIS METAXAS *(Professor of Medicine and Chairman, Second Department of Propedeutic Medicine, University of Thessaloniki Medical School, Physician-in-Chief, Agia Sophia Hospital, Thessaloniki, Greece):* Our patient suffered his first attack of severe pleurodynia at age 26. For the next 2 years, he experienced frequent abdominal pain and paroxysm in the intercostal muscles; these attacks usually were accompanied by low-grade fever that lasted 2 to 3 days. He then underwent an exploratory laparotomy and appendectomy, and during that hospitalization, moderate proteinuria was first detected. The development of ankle edema and the finding of heavy proteinuria prompted performance of a renal biopsy that revealed moderately severe renal amyloidosis. One month after biopsy, he was first admitted to our hospital. We diagnosed familial Mediterranean fever (FMF) on the basis of the positive family history, his Asia Minor origin, and clinical and laboratory studies that excluded other diseases known to cause renal amyloidosis.





Figs. 3 and 4. *Second biopsy (1980), showing heavy amyloid deposits in the glomeruli, mainly in the mesangium, producing a lobular pattern and a double contour effect, and leading to glomerular obsolesence with partial tubular atrophy.*

Familial Mediterranean fever was clearly established as a nosologic entity more than 20 years ago by Heller and coworkers [1]. However, sporadic cases of the disease had been described previously under various eponyms [2–6]. Although the disease continues to attract the interest of many investigators [7], the cause of the disease remains unknown.

Familial Mediterranean fever is characterized by a Mediterranean geographic distribution, genetic transmission in an autosomal recessive fashion, and periodic attacks that remit spontaneously. It is also characterized by the frequent development of amyloidosis in which the kidney is the organ predominantly affected. In 1972, a new feature of the disease was discovered, namely, the complete prevention or marked reduction in the frequency and severity of attacks of pain by the long-term administration of colchicine [8].

The disease is diagnosed by established clinical criteria (Table 1) and by the presence of associated, but not specific, laboratory findings (Table 2). A significant fraction of the population of Northern Greece is of Asia Minor origin; thus, patients with FMF are not rare in our region. In the patient we are discussing today, we were much aided in our diagnosis because we already had diagnosed FMF in the patient's sister. Moreover, renal amyloidosis had been documented histologically before we examined the patient. Even in the absence of the renal biopsy, however, the finding of heavy proteinuria and the frequency with which renal amyloidosis complicates FMF

would have made such a diagnosis a virtual certainty [9, 10]. The incidence of amyloidosis in patients with FMF appears to be markedly affected by ethnic background. An incidence of 60% has been recorded in Turks [10], approximately 40% in Sephardic Jews [11], and lower rates in Ashkenazic Jews and Armenians [9, 12–14]. Of 9 consecutive patients with FMF in Northern Greece, 2 had renal amyloidosis when first examined [15].

Proteinuria not due to amyloidosis has been described in patients with FMF [9, 13, 14, 16]. At present, therefore, a definitive diagnosis of amyloidosis in FMF requires histologic evidence. Derosena, Koss, and Pirani, using electron microscopy, reported the presence of amyloid fibrils in the urinary sediment of a patient with renal amyloid [17]. This procedure would, of course, provide a simple technique for diagnosing the disease but, unfortunately, this finding has not been confirmed [18]. Gingival or rectal biopsies, if they include samples from submucosal layers, are positive for amyloidosis in about 80% of patients; therefore, renal biopsy may not be necessary. In theory, the diffusely infiltrated amyloid kidney, having an altered consistency and elasticity as well as extensive involvement of the arterial and arteriolar walls, could be exceptionally prone to bleed after renal biopsy. Indeed, this patient experienced hematuria that lasted for 4 days after the second biopsy. In practice, however, renal biopsy in patients with amyloidosis has not caused special problems or complications [19–21]. Renal biopsy should be preceded by a thorough investigation of blood coagulation, because hemostatic defects such as Factor X deficiency occasionally have been described in patients with amyloidosis [22–26]. Blood coagulation in this patient remained normal with the exception of a mild but definite inhibition of platelet aggregation induced by collagen. Spontaneous perinephric bleeding due to microaneurysms of the renal vessels in FMF-associated renal amyloidosis has been described; Dor et al have reported 3 such patients [27].

At this point, I would like to ask Dr. Gavrielides to present the findings of the first renal biopsy of our patient.

DR. KLIMIS GAVRIELIDES (*Department of Pathology, Theagenion Medical Institute, Thessaloniki, Greece):* The renal parenchyma, which was satisfactorily preserved, contained 16 glomeruli, convoluted and collecting tubules, and a delicate stroma. Amyloid, present in relatively small amounts in the arterial and arteriolar wall, was quite obvious along the capillary wall of the glomerular loops (Figs. 1 and 2).

DR. METAXAS: I should add that our patient had no evidence of amyloid involvement of other organs. He had no hepatomegaly or splenomegaly. Clinical and laboratory investigation did not reveal malabsorption. Thyroid and adrenal function were intact. The cardiac echogram and the electrocardiogram were normal. These negative findings are consistent with the observation that patients with amyloidosis complicating FMF only rarely manifest evidence of intestinal, endocrine, or cardiac involvement [14, 28–30]. Apparently, the extracellular accumulation of amyloid in such sites does not produce local disturbances or functional problems until a far advanced stage of involvement is reached. For example, Berlyne and coworkers found normal adrenal cortical function in patients undergoing hemodialysis who had renal amyloidosis [28].

The problem, when the patient under discussion was first examined several years ago, was how to proceed with therapy.

Table 1. Diagnostic criteria for FMF

Brief attacks of fever (self-limited)
Brief attacks of pleuritic and/or abdominal pain
Genetic and geographic predisposition
Positive family history
Renal amyloidosis
Therapeutic effect of colchicine
Negative investigation for other causes of fever and serositis

Table 2. Nonspecific laboratory findings in FMF

Leukocytosis, neutrophilia
Increased erythrocyte sedimentation rate
Increased plasma fibrinogen
Positive C-reactive protein
Increased SAA protein

The prognosis appeared grim. Confident that the amyloidosis was secondary to the FMF, we administered colchicine, which already had been shown to produce impressive results in controlling the typical periodic attacks of FMF [8]. We administered an increasing dose that eventually reached the level of 1 mg three times daily. The clinical and laboratory course of this patient over the subsequent 5 years has been instructive and gratifying. The painful crises disappeared; proteinuria started to decrease by 8 months, and by 18 months the peripheral edema had resolved and diuretic administration was discontinued. Over the subsequent 3 years, urinary protein excretion ranged from 0.5 to 1.0 g/24 hours. The patient has returned to full employment, has a normal, 2.5-year-old child, and his wife currently is pregnant. We should note, however, that over the last 6 months, protein excretion has increased to as high as 1.8 g/24 hours, and the serum creatinine has risen from 0.8 to 1.0 mg/dl to 1.3 mg/dl. We also should note that the patient himself decreased the dose of colchicine over the last 18 months to 0.5 mg daily because he was planning to father a second child. He has remained free of painful attacks, however.

I would now like to ask Dr. Gavrielides to present the findings of the second renal biopsy, which was obtained more than 5 years after colchicine therapy was begun.

DR. GAVRIELIDES: The glomeruli were larger than usual and showed a lobular pattern, which resulted from eosinophilic deposits in the mesangium and along the capillary wall. These deposits had caused variable obliteration of the capillary lumen and had led to partial or complete sclerosis (obsolescence) of three of the glomeruli. In several instances, splitting of the basement membrane was present. Silver-methenamine impregnation revealed a double-contour configuration of the capillary wall. The convoluted tubules showed small foci of atrophy and basement membrane thickening, whereas some of the collecting tubules were dilated and contained hyaline casts. The stroma was increased and sclerosed in places but showed no inflammatory infiltrate. Walls of the arteries and arterioles were irregularly thickened, but the juxtaglomerular apparatuses were not prominent. The Congo red stain was strongly positive in the glomeruli, the arterial wall, and the tubules both under light microscopy and under polarized light.

There are both quantitative and qualitative differences between the first and second biopsies. In the second, the amyloid

deposits are more prominent and caused glomerular obsolescence and tubular atrophy with stromal increase; they also seem to be located mainly within the mesangium, producing a prominent lobulation of the glomeruli.

DR. METAXAS: The findings of the second biopsy did not totally surprise us because it is well known that amyloid, especially renal amyloid, is very resistant to proteolysis and phagocytosis. Experimental studies and clinical experience, however, have documented regression of amyloid deposits [31–33]. Triger and Joekes [34] and Waldenström [35] have presented isolated case reports in which renal amyloidosis resolved both clinically and histologically following resolution of the primary disease. In general, however, renal histologic abnormalities persist despite clinical improvement [36, 37].

The beneficial influence of colchicine on this patient's nephropathy is consistent with clinical and experimental observations. Remission of the nephrotic syndrome and stabilization of renal function have been described in a number of patients [38–40]. To our knowledge, however, in no patient has a second renal biopsy been obtained to judge the effectiveness of colchicine therapy on the histology of the kidney. Using rectal biopsy, Ravid and colleagues have observed partial resolution of amyloidosis [40]. Despite the histologic findings in the present patient, we believe that the administration of colchicine was beneficial because of the observed changes in this patient's urinary protein excretion. We have observed a similar clinical response in another patient after long-term administration of colchicine. Furthermore, it appears that colchicine may prevent the development of amyloidosis in FMF. According to Gafni, Sohar, and Zemer, 3 years after the wide adoption in Israel of the policy of administering colchicine to patients with FMF, the number of patients who manifest renal amyloidosis has decreased significantly. Whereas previously these investigators encountered approximately 6 to 12 new patients with amyloidosis per year, they have seen only one patient since the widespread use of colchicine was accepted [19]. According to these authors, colchicine did not help patients who already were uremic. In the near future we surely will have more observations on the therapeutic role of colchicine in patients with established renal amyloidosis and FMF and, possibly, in patients with other forms of secondary amyloidosis.

The ability of colchicine to prevent the experimental amyloidosis that occurs following repeated injections of casein is well known [31–33]. Colchicine has an antimitotic effect on a wide variety of cells implicated in the production of SAA, the serum protein currently considered the circulating precursor of AA protein, which forms amyloid deposits in FMF and other secondary amyloidoses [41–43].

The potential therapeutic role of colchicine in the renal amyloidosis of FMF can be assessed only when viewed in the context of the natural history of the disease. Renal amyloidosis, regardless of its cause, generally is considered one of the most malignant renal maladies because it rapidly and inexorably progresses to end-stage renal failure. Cases of spontaneous remission are extremely scarce [44, 45]. As shown in Table 3, the renal amyloidosis of FMF progresses according to a fairly predictable pattern [19]. During the initial *preclinical phase*, investigation of kidney function and urinalysis are, by definition, unrevealing. Notwithstanding, even in this phase histologic examinations can reveal amyloid in various organs; in the case of the kidney, amyloid is deposited in arteries and in the arterioles of the glomerulus. It is difficult to quantify the duration of this phase, but it probably varies considerably.

Table 3. Natural history of renal amyloidosis in FMF

Phases	Characteristics	Duration *(years)*	Range *(years)*
Preclinical	None	unknown	
Proteinuric	Mild proteinuria	3–4	2–10
Nephrotic	Full nephrotic syndrome	1–2	0.5–5
Uremic	Progressive chronic renal failure	1–1.5	0.5–6
Hemodialysis	FMF attacks ameliorated?		
	Amyloidosis of other organs		
Posttransplant	Exacerbation of FMF attacks		
	Recurrent amyloidosis in graft		

The preclinical phase is followed by a *proteinuric phase*, during which moderate proteinuria is discovered, often incidentally; once detected, the proteinuria is usually persistent. The urinary sediment is characteristically nonspecific. Occasionally the patient manifests microscopic or macroscopic hematuria [14, 46]. Histologically, renal amyloidosis involves the glomerular capillaries in a segmental fashion; the degree of involvement varies substantially among glomeruli. Electron microscopy reveals that the amyloid fibrils are deposited in the form of nodules on the endothelial side of the basement membrane. However, intramembranous amyloid deposits also are present. as are deposits on the epithelial side of the membrane; fusion of foot processes is also noted. The proteinuric phase lasts from 3 to 4 years, the range being 2 to 10 years.

In 60% to 80% of patients, a *nephrotic phase* characterized by heavy proteinuria and edema follows the proteinuric phase. Proteinuria is usually impressively severe and nonselective. Management of the hypoalbuminemia and edema is often difficult. The persistent reduction in blood volume, often complicated by diuretic-induced volume contraction, is thought to be responsible for the frequent development of renal vein thromboses. Such thrombosis starts from the small branches of the renal vein and often progresses insidiously and gradually. This complication has been observed in as many as 39% of patients [47].

Progression from the nephrotic phase to the subsequent *uremic phase* takes 1 to 2 years; the range is 0.5 to 5.0 years. During this phase, proteinuria subsides but substantial protein losses persist relative to the decrease in GFR. Hypertension is often absent; however, a substantial number of patients have hypertension during the final stage of the disease [33, 48, 49]. End-stage renal failure ensues in 12 to 18 months; the range is 6 months to 6 years. At this stage, fibrotic scarring of the glomeruli dominates the histologic picture and the kidneys are shrunken.

Over the last several years, some patients with FMF and renal amyloidosis have been managed with chronic hemodialysis and renal transplantation [28, 50–56]. Hemodialyzed patients with FMF and renal amyloidosis, however, have a survival rate 20% less than that of the general population of patients undergoing chronic dialysis [51]. Rubinger, Friedlaender, and Popovtzer have presented evidence suggesting that during hemodialysis, the clinical attacks of FMF are ameliorated [52]. Clinical problems secondary to amyloid inva-

sion of other organs such as the small intestine, thyroid gland, and heart have been observed.

Observations on patients with FMF-associated renal amyloidosis who have undergone transplantation are few. An exacerbation in the number of attacks of FMF has been described [52], as well as amyloid deposition in the graft after 6 months to 5 years [50, 53, 54, 57]. In the few reported patients, the grafts continued to function satisfactorily and the amyloid deposition involved only the interlobular arteries. These patients should not be deprived of hemodialysis and renal transplantation. Of course, colchicine should be administered prophylactically to the transplanted patients both to control the attacks of FMF and possibly to prevent amyloid deposition in the kidney and other organs.

Questions and answers

DR. NICOLAOS E. MADIAS *(Renal Service, NEMC, Boston, Mass.):* Thank you for this fine presentation. I should like to open the question-and-answer period by referring to those patients with nephrotic syndrome and renal amyloidosis secondary to chronic inflammatory processes other than FMF. In these patients, do the nephrotic syndrome and the renal amyloid deposits resolve once the inflammatory condition is either eliminated or suppressed?

DR. METAXAS: The information available on this point is quite limited. However, early work by Waldenström [35] and subsequent work by others [33, 35, 36, 58] suggests that, if the underlying inflammatory process is treated satisfactorily, significant clinical resolution of the renal amyloidosis may occur. That is, improvement or complete regression of the nephrotic syndrome and improvement in renal function are possible. Even though this clinical improvement is sometimes accompanied by partial resolution of renal amyloid deposits, the deposits generally persist and occasionally they become even more prominent. As a rule, the clinical resolution is not reflected in resolution of the renal amyloidosis. Again, in contrast to other organs (e.g., liver, spleen, lymph nodes, skin), which appear to be much more amenable to complete resolution of the amyloid deposits, the kidneys manifest an extraordinary resistance to such regression. Moreover, if the renal amyloidosis is relatively advanced, successful treatment of the underlying inflammatory condition does not appear to ameliorate the renal manifestations. I can recall 3 patients with advanced secondary amyloidosis due to chronic osteomyelitis in whom aggressive treatment of the osteomyelitis culminating in amputation did not interrupt the deterioration of renal function.

DR. MADIAS: Certain aspects of the histopathologic evolution in the patient presented today are highly reminiscent of a report of renal amyloidosis consequent to extensive infected burns [59]. In that case, serial biopsies over a 6-year period revealed that resolution of the nephrotic syndrome was attended by substantial morphologic regression of the amyloid deposits. In the latest biopsy, however, 4 of the 12 glomeruli were obsolete. As in today's patient, irregular thickening of the glomerular basement membrane and formation of a double capillary wall contour were noted, the latter reflecting deposition of basement membrane-like material encasing the amyloid deposits. These developments were thought to represent a form of scarring consequent to the amyloid deposition.

DR. ACHILLES TOURKANTONIS *(Professor of Medicine and Chairman, A' Department of Internal Medicine, Univ. of Thessaloniki Medical School, Physician-in-Chief, AHEPA Hospital, Thessaloniki):* We currently are caring for 3 patients with FMF. In these patients, colchicine at a dose of 0.5 mg daily has been effective in eliminating the febrile and painful crises. The first patient is free of evidence of nephropathy over a 4-year period of follow-up. The second patient has developed renal amyloidosis that has progressed to moderately severe renal insufficiency over a 2-year period. The third patient was discovered in 1970 and within 4 years advanced to end-stage renal disease. A renal transplant was performed but, unfortunately, 6 months later renal failure recurred. A biopsy revealed amyloid deposition in the renal allograft; subsequently, the graft was removed and the patient was returned to chronic hemodialysis.

I have three questions: *(1)* Are the number and/or the severity of the attacks of FMF related to the development of amyloidosis? *(2)* Is there a need for daily doses of colchicine higher than 0.5 mg, a dose that usually appears effective in controlling the crises of FMF? *(3)* Do you know of any experience with the administration of dimethyl sulfoxide (DMSO) in renal amyloidosis secondary to FMF?

DR. METAXAS: In regard to your first question, there does not appear to be a relationship between the number and intensity of the attacks of FMF and the subsequent development of amyloidosis. The Israelis have described two FMF phenotypes: in phenotype I, the attacks of fever and serositis appear first and amyloidosis appears later if at all; in phenotype II, amyloidosis is the first manifestation of the disease. These observations attest to a lack of correlation between clinical attacks and the development of amyloidosis. Our experience has been quite similar. Patients with frequent and severe attacks, such as the sister of today's patient, often lack evidence of renal amyloidosis; in contrast, mild forms of FMF may be complicated by renal amyloidosis. The experience has been similar in other conditions that lead to secondary amyloidosis, such as rheumatoid arthritis, osteomyelitis, and Hodgkin's disease. It would appear that in the case of FMF, other factors besides the ethnic background importantly influence the development of renal amyloidosis.

With regard to the appropriate dosé of colchicine for controlling the crises of FMF, it varies considerably in our experience and in that of others. Only a fraction of the patients respond to a daily dose of 0.5 mg, but approximately 90% respond to a dose of 2.0 mg. It has been our practice to initiate treatment with 0.5 mg of colchicine daily and increase the dose by 0.5 mg up to 2.0 mg daily until we can control the crises. The required dose of colchicine appears equally high in the pediatric population. In the case of the patient under discussion, we administered a dose as high as 3 mg daily.

Amyloid fibrils can be denatured by DMSO. I know of only two patients with FMF-induced renal amyloidosis who received DMSO treatment; the therapy was administered only after renal insufficiency developed. There was no response to treatment [60]. I think, however, that more data on this mode of treatment will be forthcoming.

DR. SOTIRIOU *(Department of Pediatrics, Univ. of Thessaloniki Medical School, Thessaloniki):* We have been caring for a 10-year-old girl with FMF, renal amyloidosis, and the nephrotic syndrome. The crises started when she was approximately 8

months old. She has had two abdominal operations and has had episodes of monoarticular arthritis. We first saw her at the age of 8 years. At that time the nephrotic syndrome was present and the presence of renal amyloidosis was documented. Since then she has been taking increasingly higher doses of colchicine—for the last 16 months at a dose of 1.25 mg daily—and she continues to experience crises of FMF; her nephrotic syndrome has increased in severity but her renal function continues to be normal.

DR. METAXAS: As I indicated, the dose of colchicine required to control the attacks of FMF in children appears similar to that in adults. Assuming that your patient is taking the medication, I would suggest that you increase the dose further. I should point out, however, that approximately 10% of the patients will not respond to even a 2 mg daily dose of colchicine. Some other patients may also continue to have an aborted form of crises despite maximal colchicine dosage.

One should be particularly careful with the use of diuretics in patients with renal amyloidosis, severe nephrotic syndrome, or hypertension. It is my impression that injudicious diuresis in such patients precipitates renal vein thrombosis.

DR. GEORGE SAKELLARIOU: *(Renal Service, Second Department of Propedeutic Medicine, Univ. of Thessaloniki Medical School, Thessaloniki):* I have two questions: First, what is the role of colchicine in the prevention of recurrent amyloidosis following renal transplantation? Second, why is arterial hypertension so rare in patients with renal amyloidosis despite even extensive parenchymal involvement?

DR. METAXAS: I strongly believe that patients with FMF and renal amyloidosis who have undergone renal transplantation should receive colchicine for the rest of their lives even though no data currently suggest that such practice prevents recurrent amyloidosis. With regard to your second question, it is an old observation that hypertension rarely occurs with renal amyloidosis even in the presence of advanced renal failure. Adrenal insufficiency had been implicated, but studies have shown that the adrenal glands of such patients respond appropriately to ACTH stimulation [28]. In my view, the relative absence of hypertension is probably related to hypovolemia secondary to the hypoalbuminemia of the nephrotic syndrome. It is also possible that amyloid infiltration of the vessels might be responsible. I should state, however, that the rarity of hypertension in renal amyloidosis probably has been overemphasized in the literature.

DR. MADIAS: I also think that hypovolemia is probably an important factor in the relative absence of hypertension in view of the fact that these patients, like those with advanced diabetic nephropathy, often continue to excrete large amounts of protein in the urine even after end-stage renal failure develops. It has been our experience, and that of others, that at least 30% of patients with advanced amyloidosis have moderate hypertension, however [14, 61].

DR. AVRAAM AVRAMIDES *(Endocrinology Department, Second Department of Propedeutic Medicine, Univ. of Thessaloniki Medical School, Thessaloniki):* What are your recommendations about the use of chronic colchicine prophylaxis in view of the potential hazard of azoospermia?

DR. METAXAS: As you know, instances of both azoospermia [62] and aneuploidy [63] have been reported in patients taking daily colchicine therapy, but vast experience suggests that these occurrences must be very rare at the doses used for FMF prophylaxis [64, 65]. Nevertheless, this matter is relevant when one is dealing with individuals who desire to have children. It has been my practice to advise such patients to discontinue the medication 2 to 3 months before attempting to conceive.

DR. MADIAS: This particular issue has been addressed by Wright and colleagues [66]. In a preliminary study, they have shown that intermittent colchicine therapy—that is, short courses of colchicine taken at the onset of the FMF attacks—was effective in aborting the FMF crises. It would appear that this therapeutic strategy deserves further investigation.

DR. MENELAOS PAPADIMITRIOU *(Chief, Renal Service, Second Department of Propedeutic Medicine, Univ. of Thessaloniki Medical School, Thessaloniki):* I should like to refer briefly to the experience in using hemodialysis to treat patients who have end-stage renal amyloidosis. Until about 5 years ago, hemodialysis programs were reluctant to accept such patients, but this policy has changed drastically. Berlyne has reported 10 patients with renal amyloidosis who were treated with chronic hemodialysis for a period of 3.5 to 5 years; the results failed to show increased mortality or a higher rate of complications in these patients as compared to the remainder of the authors' dialytic population [28]. In a recent study by Rubinger et al [53], hemodialysis appeared to ameliorate the clinical features of FMF in a small group of patients. As Dr. Metaxas already has pointed out, the European Dialysis and Transplant Association has followed more than 700 patients with amyloidosis on chronic hemodialysis [51]. The data indicate that these patients have a 20% lower survival rate than do patients without amyloidosis on chronic hemodialysis. Patients with amyloidosis appear to have greater problems with vascular access because of the poorer quality of their vessels and the greater tendency for them to develop infectious complications. Progressive amyloid involvement of other organs is, of course, another factor contributing to their limited survival. Infections and cardiac complications appear to be the leading causes of death.

Forum commentary

KEITH P. W. J. MCADAM *(Assistant Professor of Medicine, Tufts University School of Medicine; and Experimental Medicine Division, Department of Medicine, NEMC, Boston, Mass.):* Dr. Panagiotis Metaxas has reviewed most of the clinical aspects of FMF and amyloidosis relevant to this very interesting patient. I therefore shall focus on the current understanding of the pathogenesis of secondary amyloidosis and on the proteins associated with the deposition of amyloid fibrils in the tissues. Patients with secondary amyloidosis have in common a predisposing disease characterized by recurrent episodes of fever and inflammation, with neutrophil leukocytosis and elevation of the serum amyloid A protein (SAA) [67]. Familial Mediterranean fever is an excellent example of such a disease, but the conditions causing secondary amyloidosis are quite diverse in nature. They include chronic inflammatory diseases such as rheumatoid arthritis and Crohn's disease, suppurative diseases such as osteomyelitis and bronchiectasis, mycobacterial diseases including lepromatous leprosy and tuberculosis, and certain tumors such as Hodgkin's disease and renal carcinoma. Although hypergammaglobulinemia is often present, the major fibrillar protein isolated from organs infiltrated with amyloid in these diseases is the amyloid A protein (AA). The

amyloid A protein is distinct from the imunoglobulin light-chain fragments that form the major fibril protein (AL) in patients with plasma cell dyscrasias and in those with primary amyloidosis, i.e., those in whom no underlying cause can be identified. Amyloid proteins of yet different chemical types have been detected in familial Portuguese neuropathy, senile cardiac amyloid, and the endocrine tumor-associated amyloidosis such as that occuring in medullary carcinoma of the thyroid [43].

An acute-phase reactant, the concentration of SAA rises many hundredfold within a few hours of an inflammatory stimulus [68]. In serum, SAA circulates with high-density lipoprotein [69], and it has been detected in at least 6 different heterogeneous forms (SAA 1 through 6) [70]. The liver is the major site of SAA synthesis [71, 72]. Recent in-vitro studies have demonstrated synthesis of SAA in hepatocytes [73]. The polymorphism of SAA in serum may reflect the ability of other tissues, including neutrophils [74] and fibroblasts [75], to produce SAA. A soluble factor from monocytes provides the trigger for acute-phase SAA synthesis [76]. The identity of this SAA-stimulating factor is now known to be identical or very similar to endogenous pyrogen and lymphocyte-activating factor [77]. Thus, purified endogenous pyrogen, produced by activated macrophages, causes fever, stimulates hepatocytes to synthesize SAA, and activates lymphocytes to proliferate. This highly active molecule therefore may be the actual common denominator among the several diseases that cause secondary amyloidosis. It is noteworthy that patients with amyloidosis do not appear to have a prolonged SAA response after an inflammatory stimulus (McAdam, Elin, Sipe, and Wolff, unpublished observations).

Why only a minority of patients with chronic inflammatory diseases develop amyloidosis is a central issue in amyloid research. Elastase-type enzymes have been identified on the monocyte membrane [78], in the serum [79], and within neutrophil lysosomes [80], which are capable of cleaving the 11,000 to 12,000 dalton SAA to a smaller AA-sized fragment (molecular weight, 5000 to 8000). Thus, an enzyme deficiency in the catabolic pathway of SAA could, in theory, predispose some individuals to secondary amyloidosis. It is not clear whether some SAA isotypes are more prone than others to form amyloid fibrils. A nonfibrillar component, known as the pentagonal or "P" component of amyloid (AP), has been found to coexist in amyloid deposits irrespective of whether the chemical nature of the amyloid fiber was AL, AA, or AMCT (amyloid in medullary cell carcinoma of the thyroid). This component was first detected by electron microscopy of amyloid deposits as a pentagonal structure of outside diameter 90 Å with an empty inner core [81]. Antibodies to AP cross react with an alpha globulin in normal human serum (SAP) [82–84]. Because AP and SAP have an identical structure, amino acid composition, and sequence [85], they are thought to be composed of two doughnut-like structures having 5 subunits of approximately 20,000 molecular weight each [86]. This protein is distinct from SAA but has substantial sequence homology with C-reactive protein (CRP), which also has a pentagonal tertiary structure [87]. Although SAP is not an acute-phase protein in humans, it is in mice [88], and it appears to be an integral part of the glomerular basement membrane. Immunofluorescent studies indicate increased concentrations of SAP in renal lesions associated with a wide variety of nephropathies, including amyloidosis [89]. Although SAP binds to amyloid fibrils in a calcium-dependent fashion [90], it is not clear whether its presence in amyloid deposits reflects an "innocent bystander" role or whether it acts as the "magnet" that attracts amyloid proteins to the basement membrane. Other proteins including the calcium-dependent clotting factors X, IX, VII, and prothrombin also bind to amyloid fibrils in vitro [91]. This accounts for the occasional finding of a coagulopathy in patients harboring the AL form of amyloid. Splenectomy reversed Factor X deficiency in one patient with splenomegaly associated with AL [92]. When petechiae and ecchymoses occur, they generally reflect an underlying capillary fragility.

Despite our greatly increased understanding of the pathogenesis of amyloidosis, there has been relatively little progress in the clinical management of patients with this condition. Recent clinical advances include the development of a rapid, simple needle biopsy of abdominal wall subcutaneous fat [93]. This technique is reported to be as reliable as rectal biopsy for diagnosis of amyloidosis. In addition, it makes prevalence studies of amyloidosis easier [94] and allows for relatively noninvasive histologic sampling during treatment so that one can assess the efficacy of therapy. Several different histologic techniques have been developed to differentiate the chemical varieties of amyloid. Deposits of AA are identified by their sensitivity to proteolysis or potassium permanganate treatment; after such treatment, they fail to stain with Congo red [95]. Specific amyloid protein antisera are now available and have permitted the utilization of immunofluorescent and immunoperoxidase testing [96].

Treatment of the underlying disease is of utmost importance in the control of secondary amyloidosis. Colchicine has proved highly effective in preventing painful attacks in FMF and in reducing the incidence of amyloidosis in this disease. How it does this is still unclear. Experimentally, colchicine prevents the induction of amyloidosis in laboratory animals. In vitro, colchicine prevents the secretion of SAA from the liver and results in the accumulation of SAA within hepatocytes [72, 73]. Therapeutic doses of colchicine given to volunteers and to patients with amyloidosis reduce the SAA elevation caused by a standard inflammatory stimulus by at least 50%. Dosages larger than 0.5 mg three times a day generally cause diarrhea and are not tolerated long.

Apart from colchicine, the only therapeutic agent reported to be helpful in patients with secondary amyloidosis is DMSO. This remarkable solvent has been tested as a prevention and a treatment of AA disease experimentally [97, 98]. Anecdotal case reports suggest cause for optimism in patients [99, 100], although the usefulness of the drug is seriously impaired by the permeating odor of the metabolic product, dimethyl sulphide, which is excreted on the breath for up to 8 days following a single dose. One report stated that DMSO caused increased excretion of AA fibrils in the urine and suggested that reversal of disease may be possible [101]. Any claims for successful treatment of amyloidosis need to be interpreted with caution, however, in view of the occasional, well-documented spontaneous remissions. Moreover, redistribution of amyloid deposits from spleen and liver to the kidney following DMSO treatment has been observed experimentally [102]. There is evidence that biologic pathways do exist by which established amyloid deposits can be mobilized. Administration of colchicine and treat-

ment of the underlying disease might prevent new fibril formation; under these circumstances, catabolic enzymes for the AA protein, even if reduced, may be sufficient to clear the body of its secondary amyloid deposits.

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